PATENT
Attorney Docket No.: 022101-001900US
Client Ref. No.: 19094-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Gregor SAGNER et al.

Application No.: 09/823,712

Filed: March 30, 2001

For: METHOD FOR DETERMINING THE EFFICIENCY OF NUCLEIC ACID AMPLIFICATIONS

Customer No.: 41504

Confirmation No. 7485

Examiner: Suryaprabha Chunduru

Technology Center/Art Unit: 1637

DECLARATION OF CARL WITTWER, PH.D., M.D. UNDER 37 C.F.R. §1.132

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Carl Wittwer, Ph.D., M.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. in the field of Biochemistry from Utah State University in 1982 and an M.D. at the University of Michigan in 1984. I am currently a professor in the Department of Pathology at the University of Utah School of Medicine. I have been in this position since 1988. I am also a cofounder of Idaho Technology, Inc., a company supplying PCR products. A copy of my curriculum vitae is attached hereto as Exhibit A.

3. I have reviewed U.S. Patent Application No. 09/823,712 (the '712 application) as well as the office actions dated July 13, 2005 and December 23, 2005 and the Applicant's response dated October 4, 2005. I understand that the claims, as amended in the concurrently filed amendment, include a step involving determination of a non-linear continuously differentiable function of a logarithm of the initial copy number of target nucleic acid in a dilution series used for amplification as a function of the cycle number at which the signal threshold value is exceeded.

4. I understand that the Examiner has rejected the pending claims as obvious over Lowe *et al.*, WO 99/54510 in view of Wittwer et al., U.S. Patent No. 6,174,670 ("the '670 patent"). I am an inventor of the '670 patent. According to the Examiner, Lowe *et al.* describes a number of the steps of the claims of the '712 application, but does not describe determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for amplification as a function of the cycle number at which the signal threshold value is exceeded. However, the Examiner has argued that the '670 patent described "DNA monitoring at each PCR cycle by measuring melting curves and calculating copy number at each cycle utilizing a DNA-binding dye (SYBR Green I)," and concludes that the '670 patent teaches a non-linear function that would be obvious to combine with the Lowe reference

to achieve the presently claimed invention. *See*, December 23, 2006 office action, page 4, second and third paragraphs.

5. I disagree with the Examiner because the '670 patent does not describe generating a non-linear function of the logarithm of initial copy number and the cycle threshold (the cycle number at which the signal threshold value is exceeded). The Examiner correctly states that the '670 patent describes a method of DNA monitoring at each PCR cycle by measuring melting curves and calculating copy number at each cycle. However, the claimed invention involves determining a non-linear function of the logarithm of the initial concentration of nucleic acid in multiple dilutions and the cycle threshold. Monitoring amplification at each cycle, as described in the cited sections of the '670 patent, does not render it obvious to determine a non-linear function of the logarithm of initial copy number and cycle threshold.

6. The sections of the '670 patent cited by the Examiner (col. 3, lines 30-61; col. 4, line 45-63; col. 7, line 14-31; Figs. 22-23; and col. 17, lines 34-39) describe monitoring of amplification in real time (i.e., at every amplification cycle), but these sections do not teach or suggest the claimed non-linear relationship of the logarithm of initial copy number and cycle threshold. For example, the Examiner has cited col. 4, lines 45-63 of the '670 patent, which refers to a "3-dimensional spiral," for a teaching of "nonlinear functionality." *See*, December 23, 2005 Office Action, page 4, third paragraph. While the '670 patent does indeed refer to a "3-dimensional spiral," the spiral has nothing to do with the invention as currently claimed in the '712 application. Rather than teaching anything about the relation of the logarithm of initial copy number and cycle threshold, the "3-dimensional spiral" refers to measurement of temperature, time and fluorescence during each cycle of an amplification. *See*, the '670 patent, col. 4, lines 52-63. Measurement of temperature, time and fluorescence *within* an amplification does not suggest the relation of the logarithm of initial copy number and cycle threshold

because these measurements do not involve analysis of dilutions of a target nucleic acid. Indeed, the use of the term "3-dimensional" does not relate at all to a mathematical function as recited in the claims of the '712 application. Therefore the Examiner is not correct in stating that the "3-dimensional spiral" discussed in the '670 patent has anything to do with the non-linear functions recited in the claims of the '712 application.

7. Figures 22 and 23 of the '670 patent, also cited in the Examiner's rejection, do not teach or suggest determining a non-linear function of the logarithm of initial copy number and cycle threshold. Figure 22 displays real time fluorescence of an amplification of one sample. Fluorescence information from one amplification does not provide information regarding the cycle threshold values from *different* dilutions that one of skill in the art would use to determine a function between the logarithm of copy number and cycle threshold.

8. Figure 23 displays real time fluorescence in relation to cycle number for different dilutions of a target nucleic acid. However, determining a non-linear function of the logarithm of initial copy number and cycle threshold is not suggested in Figure 23. Moreover, it was commonly assumed before the filing of the '712 application that a *linear* function of the logarithm of initial copy number to cycle threshold should be determined. This is illustrated, for example, in the Lowe *et al.* reference (page 5, lines 7-8 and Figure 1B), each of which use *linear* regression to determine a *linear* function between the logarithm of initial copy number and cycle threshold, and in Figures 24, 26, and 28 of the '670 patent, which suggests good fit to a linear function. Nothing in the art the Examiner has cited contradicts this common assumption, i.e., that one of skill in the art should generate a *linear* function of the logarithm of initial copy number and cycle threshold.

9. Column 40, lines 14-24 of the '670 patent notes a decrease in reaction efficiency when initial copy number is 100 copies or below. However, this result was interpreted as a result of primer dimers and nonspecific amplification (col. 40, lines 24), and the data from samples with low initial copy number were normalized using a value generated from melting peak integration (col. 40, lines 40-47). This is illustrated, for example, in Figure 42A, which illustrates the original data, and Figure 42D, which shows the normalized data. The normalized data presented in Figure 42D from the various dilutions are shown as amplification curves that have approximately equal spacing, even at low dilutions, in contrast to the original data displayed in Figure 42A. While not graphically represented as a logarithm of the copy number of target nucleic acid as a function of the cycle number at which the signal threshold is exceeded, this normalization process as described in col. 40 and Figure 42, would tend to linearize such a function.

10. In view of the forgoing, it is my scientific opinion that the claimed invention in the '712 application is not obvious. The combination of the Lowe *et al.* reference and the '670 patent does not teach or suggest a step of determining a non-linear continuously differentiable function of logarithm of initial copy number as a function of cycle threshold.

Date: April 6, 2006 By: _____

Carl Wittwer, Ph.D., M.D.

Curriculum Vitae

I. PERSONAL DATA

Carl Thomas Wittwer
Born March 8, 1955, Lansing, MI, USA
US Citizenship
Ethnicity: White
SSN: 366-64-4751

II. EDUCATION/LICENSURE

Middlebury College, Middlebury, Vermont College Scholar	1973-1975
Utah State University, Logan, Utah BS Chemistry, PhD Biochemistry	1975-1978 1980-1982
University of Michigan, Ann Arbor, Michigan MD	1978-1980 1982-1984
State of Utah Medical License	1984
University of Utah, Salt Lake City, UT, Residency	1984-1988
Board Certified in Anatomic and Clinical Pathology	1988
New York State Certification for genetic testing, molecular oncology, immunohematology, and cellular immunology	1992-

III. PROFESSIONAL EXPERIENCE

A. Full Time Positions:

Professor Department of Pathology University of Utah School of Medicine	2000-
Associate Professor Department of Pathology University of Utah School of Medicine	1994-2000
Assistant Professor Department of Pathology University of Utah School of Medicine	1988-1994

B. Part Time Positions:

Chief Science Officer/Vice President for Research Idaho Technology Salt Lake City, UT	1990-
Medical Director Myriad Diagnostics, Salt Lake City, UT	1993-1996

Adjunct Assistant Professor Department of Nutrition and Food Science Utah State University	1985-2000
NIH Study Section, Biological and Physiological Sciences Special Emphasis Panel, SSS-Y	1997-
NCI Study Section and Site Visit Reviewer	1998-
Molecular consultant (Various companies)	1999-

C. Editorial Experience:

1. Clinical Chemistry, Board of Editors, 2000-

2. Clinical Chemistry, Associate Editor, 2002-

3. Rapid Cycle Real-Time PCR -- Methods and Applications. S Meuer, **C Wittwer**, K Nakaguwara, eds., Springer, Berlin, 2001.

4. Molecular Testing in Laboratory Medicine: Selections from Clinical Chemistry, 1998-2001, with Annotations and Updates. DE Bruns, YMD Lo, and **CT Wittwer**, eds., AACC press, Washington, DC, 2002.

5. Rapid Cycle Real-Time PCR – Methods and Applications: Microbiology and Food Analysis. U. Reischl, **C Wittwer**, and F. Cockerill, eds., Springer, Berlin, 2002.

6. Rapid Cycle Real-Time PCR – Methods and Applications: Genetics and Oncology. Dietmaier, **C. Wittwer**, and Sivasubrananian, eds., Springer, Berlin, 2002.

7. Rapid Cycle Real-Time PCR – Quantification. **C. Wittwer**, M. Hahn, and K Kaul eds., Springer, Berlin, 2004.

4. Research article referee for the following journals:
Am. J. Path.
Analytical Biochemistry
Anal. Chem.
BioTechniques
Biochim. Biophys. ACTA
Clinical Chemistry
Cytometry
Human Mutation
J. Mol.Diag.
Nature Med.
Nucl. Acids Res,
Proc. Natl. Acad. Sci.

D. Research Awards:

Instrumentation for quantitative rapid cycle PCR, Technology Innovation Grant. University of Utah Research Foundation., Principal Investigator, 7/94-6/96, $90,000.

Continuous monitoring of rapid cycle PCR. NIH STTR Phase I and Phase II Grants, Principal Investigator, 9/94-9/98, $600,000.

Temperature cycling by adiabatic compression. Biomedical Engineering Grant. Whitaker Foundation, Principal Investigator, 12/95-11/98, $210,000.

Fluorescent PCR techniques. Idaho Technology, Principal Investigator, 7/97-12/02, $950,000.

Homogeneous multiplex PCR by fluorescence and Tm. NIH STTR Phase I and II Grant, Principal Investigator, 4/1/99-2/03, $620,000.

Single-Labeled Probes for Real-Time PCR. Technology Commercialization Project. University of Utah Research Foundation, Principal Investigotor, 7/02-6/03, $35,000.

Fluorescenct PCR techniques. Idaho Technology, Principal Investigator, 1/03-12/07, $1,650,000.

Center for Homogeneous DNA Analysis. State of Utah Centers of Excellence Grant, Principal Investigator, 7/03 – 6/08, $150,000/year pending approval each year.

SNP analysis without probes. Technology Commercialization Project. University of Utah Research Foundation, Principal Investigator, 7/03-6/05, $70,000.

Homogeneous mutation scanning. NIH Fast Track STTR, Principal Investigator, 8/04-1/07, $850,000.

A system for rapid PCR, mutation scanning and genotpying. NIH Fast Track STTR, Principal Investigator, 3/05-9/07, $850,000.

E. Patents and Copyrights:

DNALYSIS - DNA content and cell cycle analysis software, copyright 1989, University of Utah.

US Patent 5,455,175. Automated Rapid Temperature Cycling Device, University of Utah, 1995.

US Patent 5,935,522. On-line DNA analysis system with rapid thermal cycling, U. of Utah, 1999.

US patent 6,140,054. Multiplex genotyping using fluorescent hybridization probes, Univ. of Utah, 2000.

US patent 6,174,670. Monitoring amplification of DNA during PCR, U. Utah, 2001.

US patent 6,197,520. Solution-based color compensation adjusted for temperature and electronic gains, Univ. of Utah, 2001.

US patent 6,232,079. PCR method for nucleic acid quantification utilizing second or third order rate constants, U. Utah, 2001.

US patent 6,245,514. Fluorescent donor-acceptor pair with low spectral overlap, U. Utah, 2001.

US patent 6,303,305. Method of quantification of an analyte, U. Utah, 2001.

U.S. Patent 6,387,621. Automated analysis of real-time nucleic acid amplification, Univ. of Utah, 2002.

US patent 6,472,156. Homogeneous multiplex hybridization analysis by color and Tm, Univ. of Utah, 2002.

US patent 6,503,720. Method for quantification of an analyte, Univ. Utah, 2003.

US patent 6,569,627. Monitoring hybridization during PCR using SYBR.™. Green I, Univ. of Utah, 2003.

US patent 6,635,427. Single-labeled oligonucleotide probes for homogeneous nucleic acid sequence analysis, Univ of Utah, 2003.

US patent 6,730,501. Multi-test analysis of real-time nucleic acid amplification, Univ. Utah, 2004.

US patent 6,753,141. Simultaneous screening and identification of sequence alterations from amplified target, U. Utah, 2004.

US patent 6,787,338. Method for rapid thermal cycling of biological samples. U. Utah, 2004.

NZ patent 333136. Monitoring hybridization during PCR. U. Utah. 2000.

NZ patent 333137. System and method for carrying out and monitoring biological processes. U. Utah, 2000.

NZ patent 502323. Monitoring hybridization during PCR. U. Utah, 2002.

Australian patent 726501. Monitoring hybridization during PCR. U. Utah., 2001.

Australian patent 727296. systems and methods for monitoring for DNA amplification by fluorescence, U. Utah, 2001.

Australian patent 729644. System and method for monitoring PCR processes, 2001.

Japanese Patent No. 3670967. Multiplex Genotyping Using Fluorescent Hybridization Probes.

Published US patent application 20020151039 DNA amplification using electrolyte conductance heating and temperature monitoring, Univ. Utah.

Published US patent application. 20030165867 Multi-test analysis of real-time nucleic acid amplification, Univ. Utah.

Published US patent application. 20030224434 Genotyping by amplicon melting curve analysis, Univ. Utah.

Published US patent application. 20040002098 Monitoring amplification with fret probes, Univ. Utah.

Published US patent application. 20040033518 Characterization of single stranded nucleic acids by melting analysis of secondary structure using double strand-specific nucleic acid dye, Univ. Utah.

Published PCT patent application WO 2004/038038 A2. Amplicon melting analysis with saturation dyes, Univ. of Utah and Idaho Technology.

Published US patent application 20040265892 Mehod for rapid cycling of biological samples., Univ. Utah.

Published US patent application 20050034493 Mehod for rapid cycling of biological samples., Univ. Utah.

IV. HONORS AND AWARDS

Greenwood Memorial Scholarship in Biochemistry	1980
Cleveland Clinic Award for Medical Research	1984
FDA Physician Sponsor (Investigational New Drug Trials) for carnitine (IND #25,201) and pantethine (IND #25,416).	1986-2000
Young Investigators Award, ACLPS	1987
International presentations:	
Korea: Flow cytometry	1987-1989
Korea, Taiwan: Rapid PCR techniques	1992, 1994
Germany: Fluorescent PCR Techniques	1997-
Norway: Fluorescent PCR Techniques	1999
Outstanding Teaching Award, Clinical Pathology, Univ. of Utah	1994, 1996
Franklin Jefferson Award for Science, Technology, and Innovation (NIH STTR), The Small Business High Technology Institute.	1999
Scott and Dorothy Watkins Endowed Chair in Pathology Honoring Ernst Eichwald	1999-2002
Gordon Jensen Pathfinder Award Small Business/Inventors Conference, Salt Lake City, UT	2002
Governor's Medal for Science and Technology State of Utah	2003
Stoel Rives Innovation Award, First Place (BioTechnology) High Resolution Mutation Scanning	2004
AACC award for Outstanding Contributions to Clinical Chemistry in a Selected Area of Research	2004
Outstanding Speaker Award, AACC	2003, 2004
Innovation Award, Technology Transfer University of Utah	2004
Technical Advancement Award IQLM (International Quality in Loboratory Management) CDC/Atlanta	2005
IFCC-Abbott Award for Significant	2005

Contributions to Molecular Diagnostics
IFCC/AACC

V. ADMINISTRATIVE EXPERIENCE

Medical Director Flow Cytometry Associated and Regional University Pathologists	1988-
Associate Director HLA Laboratory University of Utah	1988-1992
Technical Vice President of New Technology Associated Regional and University Pathologists Salt Lake City, UT 84108	1990-
Medical Director Molecular Diagnostics Associated and Regional University Pathologists	1992-1996
Medical Director Advanced Technology Group Associated and Regional University Pathologists	2001-

VIII. MEMBERSHIP IN PROFESSIONAL SOCIETIES

Society for Analytical Cytology
Association for Molecular Pathology
Academy of Clinical Laboratory Physicians and Scientists

IX. TEACHING RESPONSIBILITIES/ASSIGNMENTS

Pathology resident rotation in Flow Cytometry/ Molecular Pathology, 4-5 residents/year	1988-1999
Mentor to Resident and Fellow Research Projects with seven abstracts receiving Young Investigator Awards	1988-
Medical and graduate student guest lectures on flow cytometry, HLA typing, and DNA diagnostics.	1988-
Mentor for visiting fellows from Korea and Singapore	1994-
Major professor for students in Nutrition, Medical Laboratory Science and BioMedical Engineering.	
Panothenate-p-nitroanilide as a substrate for pantetheinase assay, Robert T. Davidson, Nutrition, Utah State University	1994

Rapid Cycle PCR for diagnosis of fragile X Syndrome, H. S. Lee, Med. Lab Sci, U of U.	1995
Continuous monitoring of the PCR for hepatitis B Virus: detection and quantification., Doug Searles, Med. Lab Sci., U of U.	1997
Fuzzy cluster image analysis of bone marrow morphology, R. K. Wang, Med. Lab Sci, U of U.	1998
Low temperature capillary fluorimeter for conformational analysis of nucleic acids, Wade Dummer, BioMedical Engineering, U of U	2000
Quenching of fluorescently-labeled probes upon hybridization Cameron Gundryt, Med Lab Sce, U of U	2003
ApoE genotyping by multiplex unlabeled probe melting analysis Matt Poulson, Med Lab Sci., U of U	2005
Genotyping four thrombophilia SNPs by multiplex small amplicon Melting without probes. Mike Seipp, Med Lab Sci, U of U	2006

PEER REVIEWED PUBLICATIONS:

1. Wyse BW, **CT Wittwer**, RG Hansen. Radioimmunoassay for pantothenic acid in blood and other tissues. Clin. Chem. 25:108-111, 1979.

2. **Wittwer CT**, BW Wyse, RG Hansen. Assay of the enzymatic hydrolysis of pantetheine. Anal. Biochem. 122:213-222, 1982.

3. **Wittwer CT**, Burkhard, K Ririe, R Rasmussen, J Brown, BW Wyse, RG Hansen. Purification and properties of a pantetheine-hydrolyzing enzyme from pig kidney. J. Biol. Chem. 258:9733-9738, 1983.

4. **Wittwer CT**, WA Gaul, JD Butler, M Zatz, JG Thoene. Metabolism of pantethine in cytinosis. J. Clin. Invest. 76:1665-1672, 1985.

5. Wyse BW, RG Hansen, CT Windham, **CT Wittwer**. The status of human nutrition and agricultural productivity. J. Home Econ. 78:19-24, 1986.

6. Gleeson JM, **CT Wittwer**, CA Schipke, DE Wilson. Effect of carnitine and pantethine on the metabolic abnormalities of acquired total lipodystrophy. Curr. Ther. Res. 41:83-88, 1987.

7. **Wittwer CT**, AM Smith, KO Ash, CW DeWitt. False-positive antibody tests for human immunodeficiency virus in transplant patients with antilymphocyte antibodies. Transplantation 44:843-844, 1987.

8. **Wittwer CT**, CP Graves, MA Peterson, E Jorgensen, JG Thoene, BW Wyse, CT Windham, RG Hansen. Pantetheine lipomodulation: evidence for cysteamine mediation in vitro and in vivo. Atherosclerosis 68:41-49, 1987.

9. Rabkin MS, CR Kjeldsberg, EG Hammond, **CT Wittwer**, B Nathwani. Clinical, ultrastructural, immunohistochemical, and DNA content analysis of lymphomas having features of interdigitating reticulum cells. Cancer 61:1594-1601, 1988.

10. Rabkin MS, **CT Wittwer**, CR Kjeldsberg, MW Piepkorn. Flow cytometric DNA-content analysis of histiocytosis X. Am. J. Path. 131:283-289, 1988.

11. **Wittwer CT**, MR Bristow, EM Gilbert, DG Renlund, JB O'Connell, CW DeWitt. OKT3 therapy as a cause of high panel reactive antibodies in serum using standard microcytotoxicity techniques. Transplantation 45:832-834, 1988.

12. Pearson SD, **CT Wittwer**, KO Ash. Evaluation of three commercial kits for the confirmation of antibodies to human immunodeficiency virus (HIV-1). Clin. Chem. 34:1930, 1988.

13. **Wittwer CT**, C Schweitzer, J Pearson, WO Song, CT Windham, BW Wyse, RG Hansen. Enzymes for liberation of pantothenic acid in blood: use of plasma pantetheinase. Am. J. Clin. Nutr. 50:1072-1078, 1989.

14. **Wittwer CT**, GC Fillmore, DR Hillyard. Automated polymerase chain reaction in capillary tubes with hot air. Nucl. Acids Res. 17:4353-4357, 1989.

15. **Wittwer CT**, WA Knape, MR Bristow, EM Gilbert, DG Renlund, JB O'Connell, CW DeWitt. The quantitative flow cytometric plasma OKT3 assay - its potential application in cardiac transplantation. Transplantation 48:533-535, 1989.

16. Song WO, A Smith, **CT Wittwer**, BW Wyse, RG Hansen. Determination of plasma pantothenic acid by indirect enzyme linked immunosorbent assay. Nutr. Res. 10:439-448, 1990.

17. Rabkin MS, CR Kjeldsberg, **CT Wittwer**, J Marty. A comparison study of two methods of peanut agglutinin staining with S-100 immunostaining in twenty-nine cases of histiocytosis X (Langerhans' cell histiocytosis). Arch. Path. Lab. Med. 114:511-515, 1990.

18. Holden JA, DH Rolfson, **CT Wittwer**. Human DNA topoisomerase II: evaluation of enzyme activity in normal and neoplastic tissues. Biochemistry 29:2127-2134, 1990.

19. **Wittwer CT**, GC Fillmore, DJ Garling. Minimizing the time required for DNA amplification by efficient heat transfer to small samples. Anal. Biochem. 186:328-331, 1990.

20. **Wittwer CT**, S Beck, M Peterson, R Davidson, DE Wilson, RG Hansen. Mild pantothenate deficiency in rats elevates serum triglycerides and free fatty acids. J. Nutr., 120:719-725, 1990.

21. Rabkin MS, **CT Wittwer**, VR Soong. Flow cytometric DNA content analysis of a case of pilomatrix carcinoma showing multiple recurrences and invasion of the cranial vault. J. Am. Acad. Derm., 23:104-108, 1990.

22. Hammond EH, **CT Wittwer**, J. Greenwood, WA Knape, RL Yowell, RL Menlove, C Craven, DG Renlund, MR Bristow, CW DeWitt, JB O'Connell. Relationship of OKT3 sensitization and vascular rejection in cardiac transplant patients receiving OKT3 rejection prophylaxis. Transplantation 50:776-782, 1990.

23. Benachenhow D, M Cader, H Szu, L Medsker, **C Wittwer**, D Garling. AIDS viral DNA amplification by polymerase chain reaction employing primers selected by AI expert system and an ART neural network. Proceedings of the 3rd Annual IEEE Symposium on Computer-Based Medical Systems 3:504-511, 1990.

24. **Wittwer, CT** and DJ Garling. Rapid Cycle DNA Amplification. BioTechniques, 10:76-83, 1991.

25. Smith, JA, AD Hernandez, **CT Wittwer**, JM Avent, J Greenwood, EH Hammond and RG Middleton. Long-term follow-up after radical prostatectomy. Urol. Clinics N. Amer. 18:473-476, 1991.

26. O'Connell, JB, DG Renlund, EH Hammond, **CT Wittwer**, RL Yowell, CW DeWitt, KW Jones, WA Gay, RL Menlove and MR Bristow. Sensitization to OKT3 monoclonal antibody in heart transplantation: correlation with early allograft loss. J. Heart Lung Transplant., 10:217-221, 1991.

27. Hopfenbeck, JA, JA Holden, **CT Wittwer** and CR Kjeldsberg. Digoxigenin-labeled probes amplified from genomic DNA detect T-cell gene rearrangements. Am. J. Clin. Path., 97: 638-644, 1992.

28. Kershisnik, MM, AS Knisely, C-CJ Sun, JM Andrews, **CT Wittwer**. Cytomegalovirus infection, fetal liver disease, and neonatal hemochromatosis. Human Pathology, 23: 1075-80, 1992.

29. Segal, GH, **CT Wittwer**, AJ Fishleder, MH Stoler, RA Tubbs, CR Kjeldsberg. Identification of monoclonal B cell populations by rapid cycle PCR: a practical screening method for the detection of immunogolbulin gene rearrangements. Amer. J. Path., 141:1291-7, 1992.

30. Weis, JH, SS Tan, BK Martin, **CT Wittwer**. Detection of rare mRNA species via quantitative RT-PCR. Trends in Genetics, 8:263-4, 1992.

31. Holden, JA, DH Rolfson, **CT Wittwer**. The distribution of immunoreactive topoisomerase II protein in human tissues and neoplasms. Oncology Research, 4:157-66,1992.

32. **Wittwer, CT**, BC Marshall, GB Reed, JL Cherry. Rapid cycle allele-specific amplification: studies with the cystic fibrosis ΔF508 locus. Clinical Chemistry, 39:804-809, 1993.

33. Albro, J, KD Bauer, CL Hitchcock, **CT Wittwer**. Improved DNA content histograms from formalin-fixed paraffin-embedded liver tissue by proteinase K digestion. Cytometry, 14:673-678, 1993.

34. Hammond, E, R Yowell, J Greenwood, L Hartung, D Renlund, **CT Wittwer**. Prevention of adverse clinical outcome by monitoring of cardiac transplant patients for murine monoclonal CD3 antibody (OKT3). Transplantation, 55:1061-1063, 1993.

35. Lee HS and **CT Wittwer**. Detection of hepatitis B virus (HBV) by polymerase chain reaction (PCR). Korean J. Clin. Path. 13:617-623, 1993.

36. Molot RJ, TC Meeker, **CT Wittwer**, SL Perkins, GH Segal, AS Masih, RC Braylan, CR Kjeldsberg. Antigen expression and PCR amplification of mantle cell lymphomas. Blood, 83:1626-1631,1994.

37. Adleberg JM and **C Wittwer**. Use of the polymerase chain reaction in the diagnosis of ocular disease. Curr. Opin. Ophthal. 6:80-85, 1995.

38. Lingenfelter B, T Fuller, L Hartung, **CT Wittwer**. HLA-B27 typing by flow cytometry. Cytometry (Clin. Appl. Cytometry), 22:146-149, 1995.

39. Lim LC, GM Segal, **CT Wittwer**. Detection of bcl-1 gene rearrangement and B-cell clonality in mantle cell lymphoma using formalin-fixed, paraffin-embedded tissues. Am. J. Clin. Path., 104: 689-695, 1995.

40. Mouritsen CL, **CT Wittwer**, CM Litwin, L Yang, JJ Weis, TB Martins, TD Jaskowski, HR Hill. Polymerase chain reaction detection of lyme disease: correlation with clinical manifestations and serologic responses. Am. J. Clin. Path., 105:647-654, 1996.

41. Florell SR, JJ Townsend, EC Klatt, TJ Pysher, CM Coffin, **CT Wittwer**, DH Viskochil. Aprosencephaly and cerebellar dysgenesis in sibs. Am. J. Med. Genet. 63:542-548, 1996.

42. Endo M, PG Beatty, TM Vreeke, **CT Wittwer**, SP Singh, Parker CJ. Syngeneic bone marrow transplantation without conditioning in a patient with paroxysmal nocturnal hemoglobinuria: in vivo evidence that the abnormal stem cells have a survival advantage. Blood, 88:742-750, 1996.

43. Segal GH, CE Hussey, and **CT Wittwer**. PCR for T-cell rearrangements. Diag. Mol. Path., 5:297-298, 1996.

44. **Wittwer CT**, MG Herrmann, AA Moss, RP Rasmussen. Continuous fluorescence monitoring of rapid cycle DNA amplification. BioTechniques, 22:130-138, 1997.

45. **Wittwer CT**, KM Ririe, RV Andrew, DA David, RA Gundry, UJ Balis. The LightCycler™: a microvolume, multisample fluorimeter with rapid temperature control. BioTechniques, 22:176-181, 1997.

46. Ririe KM, RP Rasmussen, and **CT Wittwer**. Product differentiation by analysis DNA melting curves during the polymerase chain reaction. Anal. Biochem, 245:154-160, 1997.

47. Swerdlow H, BJ Jones, and **CT Wittwer**. Fully automated DNA reaction and analysis in a fluidic capillary instrument. Anal. Chem., 69:848-855, 1997.

48. Mouritsen CL, **CT Wittwer**, G Reed, TM Khan, TB Martins, TD Jaskowski, CM Litwin, and HR Hill. Detection of Epstein-Barr viral DNA in serum using rapid-cycle PCR. Biochem. Mol. Med., 60:161-168, 1997.

49. Lay MJ and **CT Wittwer**. Real-time fluorescence genotyping of factor V Leiden during rapid cycle PCR. Clin. Chem.43:12, 2262-2267, 1997.

50. Bernard PS, MJ Lay, **CT Wittwer**. Integrated amplification and detection of the C677T point mutation in the methylenetetrahydrofolate reductase gene by fluorescence resonance energy transfer and probe melting curves. Anal. Biochem., 255:101-107, 1998.

51. Morrison TB, JJ Weis, and **CT Wittwer**. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. BioTechniques, 954-962, 1998.

52. Bernard PS, RS Ajioka, JP Kushner, and **CT Wittwer**. Homogeneous multiplex genotyping of hemochromatosis mutations with fluorescent hybridization probes. Am. J. Pathol., 153:1055-1061, 1998.

53. Pritham GH and **CT Wittwer**. Continuous fluorescent monitoring of PCR. J. Clin. Lig. Assay, 21(4):404-412, 1998.

54. Lyon E, A Millson, T Phan, and **CT Wittwer**. Detection and identification of base alterations within the region of factor V Leiden by fluorescent melting curves. Mol. Diag., 3:203-210, 1998.

55. Bohling S, TC King, **CT Wittwer**, and KSJ Elenitoba-Johnson. Rapid simultaneous amplification and detection of the MBR/JH chromosomal translocation by fluorescence melting curve analysis. Am. J. Pathol., 154: 97-103, 1999.

56. Bohling S, **CT Wittwer**, TC King, KSJ Elelitoba-Johnson. Fluorescence melting curve-based analysis for the detection of the bcl-1/JH translocation in mantle cell lymphoma. Lab. Invest. 79:337-345, 1999.

57. Hussey CE, E Lyon, A Millson, MJ Lay, **CT Wittwer**, and GH Segal. A rapid, practical PCR-based approach for the detection of PML/RAR-alpha gene fusion in acute promyelocytic leukemia. Am. J. Clin. Path., 112:256-262, 1999.

58. Bernard PS, GH Pritham, **CT Wittwer**. Color multiplexing hybridization probes using the apolipoprotein E locus as a model system for genotyping. Anal. Biochem., 273:221-228, 1999.

59. Gundry CN, PS Bernard, MG Herrmann, GH Reed, and **CT Wittwer**. Rapid F508del and F508C assay using fluorescent hybridization probes, Genetic Testing, 3:365-370, 1999.

60. Bernard PS, **CT Wittwer.** Homogeneous amplification and variant detection by fluorescent hybridization probes. Clin. Chem., 46:147-148, 2000.

61. DeSilva D, **CT Wittwer**. Monitoring hybridization during PCR, J. Chromatog. B: 741:3-13, 2000.

62. Herrmann M, S Dobrowolski, **CT Wittwer**. Beta-globin genotyping by multiplexing probe Tm and color. Clin. Chem., 46:425-428, 2000.

63. Millson AS, FL Spangler, **CT Wittwer**, E Lyon. Comparison of automated short tandem repeat and manual variable number of tandem repeat analysis of chimerism in bone marrow transplant patients. Diag. Mol. Path, 9:91-97 , 2000.

64. Heap DM, MG Hermann, **CT Wittwer**. PCR amplification using electrolytic resistance for heating and temperature monitoring. BioTechniques, 1006-1012, 2000.

65. Brown, M and **CT Wittwer**. Flow cytometry: principles and clinical applications in hematology. Clin. Chem., 46:8(B), 1221-1229, 2000.

66. Elenitoba-Johnson, KSJ, SD Bohling, **CT Wittwer** and TC King. Multiplex PCR by multicolor fluorimetry and fluorescence melting curve analysis. Nature Med., 7:249-253, 2001.

67. Crockett AO and **CT Wittwer**. Fluorescein-labeled oligonucleotides for real-time PCR: using the inherent quenching of deoxyguanosine nucleotides. Anal. Biochem., 290:89-97, 2001.

68. Lyon E, A Millson, MC Lowery, R Woods, and **CT Wittwer**. Quantification of HER2/neu gene amplification by competitive PCR using fluorescent melting curve analysis, Clin. Chem., 844-851, 2001.

69. von Ahsen, N, **CT Wittwer**, and E Schutz. Oligonucleotide melting temperatures under PCR conditions; nearest neighbor corrections for [Mg++], [dNTPs], and [DMSO] with comparison to alternate empirical formulas. Clin. Chem., 47:1956-1961, 2001.

70. **Wittwer CT**, MG Herrmann, CN Gundry, KSJ Elenitoba-Johnson. Real-time multiplex PCR assays. Methods, 25:430-442, 2001,

71. Bernard, PS, **CT Wittwer.** Real-time PCR technology for cancer diagnostics. Clin. Chem. 48:1178-1185, 2002

72. Millward, H, W Samowitz, **CT Wittwer**, PS Bernard. Homogeneous amplification and mutation scanning of the p53 gene using fluorescent melting curves. Clin. Chem. 48:1321-1328, 2002.

73. Gundry CN, JG Vandersteen, GH Reed, RJ Pryor, J Chen, and CT Wittwer. Amplicon melting analysis with labeled primers: A closed-tube method for differentiating homozygotes and heterozygotes. Clin. Chem. 49:396-406, 2003.

74. Erali M, B Schmidt, E Lyon, and **CT Wittwer**. Evaluation of electronic microarrays for genotyping Factor V, Factor II and MTHFR. Clin. Chem., 49:732-9 , 2003.

75. **Wittwer CT**, GH Reed, CN Gundry, JG Vandersteen, and RJ Pryor. High-Resolution Genotyping by Amplicon Melting Analysis using LC Green, Clin. Chem., 49:853-60, 2003.

76. Millson A, Suli A, Hartung L, Kunitake S, Bennett A, Nordberg MC, Hanna W, **Wittwer CT**, Seth A, Lyon E. Comparison of Two Quantitative Polymerase Chain Reaction Methods for Detecting HER2/neu Amplification. J Mol Diagn. 5:184-190, 2003.

77. Garcia-Villalba P, Denkers ND, **Wittwer CT**, Hoff C, Nelson RD, Mauch TJ. Real-time PCR quantification of AT1 and AT2 angiotensin receptor mRNA expression in the developing rat kidney. Nephron Exp Nephrol.; 94: e154-9, 2003.

78. Liew M, Erali M, Page S, Hillyard D, **Wittwer C**. Hepatitis C genotyping by denaturing high-performance liquid chromatography. J Clin Microbiol. 42:158-63, 2004.

79. Willmore BS, Holden JA, Zhou L, Tripp S, **Wittwer CT**, Layfield LJ. Dectection of c-kit activating mutations in gastrointestinal stromal tumors by high-resolution amplicon melting analysis. Am. J. Clin. Path. 122:206-16, 2004.

80. Zhou L Vandersteen J, Wang L, Fuller T, Taylor M, Palais B, **Wittwer CT**. High-resolution DNA melting curve analysis to establish HLA genotypic identity. Tissue Antigens, 64:156-164, 2004.

81. Liew M, Pryor R, Palais R, Meadows C, Erali M, Lyon E, **Wittwer CT**. Genotyping of single nucleotide polymorphisms by high-resolution melting of small amplicons. Clin. Chem 2004;50:1156-64.

82. Zhou L, Myers AN, Vandersteen JG, Wang L, **Wittwer CT**. Closed-Tube Genotyping with Unlabeled Oligonucleotide Probes and a Saturating DNA Dye. Clin Chem. 2004;50:1328-35

83. Reed GH, **Wittwer CT**. Sensitivity and specificity of SNP scanning by high-resolution melting analysis. Clin. Chem 2004;50:1748-54.

84. Margraf RL, Erali M, Liew M, **Wittwer CT**. Genotyping hepatitis C virus by heteroduplex mobility analysis using temperature gradient capillary electrophoresis. J Clin Microbiol. 2004;42:4545-51.

85. Margraf RL, Page S, Erali M, **Wittwer CT**. Single-tube method for nucleic acid extraction, amplification, purification, and sequencing. Clin Chem. 2004;50:1755-61.

86. Seipp MT, Erali M, Wies RL, **Wittwer CT**. HLA-B27 typing: evaluation of an allele-specific PCR melting assay and two flow cytometric antigen assays. Cytometry B Clin Cytom, 2005;63:10-5.

87. Odell ID, Cloud JL Seipp M, and **Wittwer CT**. Rapid species identification within the *Mycobacterium chelonae-abscessus* group by high-resolution melting of hsp65 PCR products. Am J Clin Path 2005;123:96-101.

88. Graham R, Liew M, Meadows C, Lyon E, **Wittwer CT**. Distinguishing different DNA heterozygotes by high-resolution melting. Clin Chem 2005;51:1295-8.

89. Chou LS, Lyon E, **Wittwer CT**. A comparison of high-resolution melting analysis to denaturing high performance liquid chromatography for mutation scanning: cystic fibrosis transmembrane conductance regulator gene as a model. Am J Clin Path, 2005;124:330-338.

90. Palais RA, MA Liew, **Wittwer CT**. Quantitative heteroduplex analysis for single nucleotide polymorophism genotyping, Anal Biochem, 2005; 346:167-75.

91. Lewis TB, Robison JE, Bastien R, Milash B, Boucher K, Samlowski WE, Leachman SA, Dirk Noyes R, **Wittwer CT**, Perreard L, Bernard PS. Molecular classification of melanoma using real-time quantitative reverse transcriptase-polymerase chain reaction. Cancer. 2005;104:1678-86.

92. Zhou L, Wang L, Palais R, Pryor R, **Wittwer CT**. High-resolution melting analysis for simultaneous mutation scanning and genotyping in solution. Clin Chem 2005;51:1770-7.

93. Chou LS, Meadows C, **Wittwer CT**, Lyon E. Unlabeled oligonucleotide probes modified with locked nucleic acids for genotyping by melting analysis. BioTechnqiues, 2005;39:644, 646, 648.

94. Margraf RL, Mao R, Highsmith WE, Holtegaard LM, **Wittwer CT**. Mutation scanning of the RET proto-oncogene using high resolution melting analysis. Clin Chem 2006;52:138-41.

95. Liew M, Nelson L, Margraf R, Mitchell S, Erali M, Mao R, Lyon E, **Wittwer CT**. Genotyping of human platelet antigens 1-6 and 15 by high-resolution amplicon melting and conventional hybridization probes. J Mol Diag, 2006;8:97-104.

96. Margraf RL, Mao R, **Wittwer CT**. Masking selected sequence variation by incorporating mismatches into melting analysis probes. Human Mut 2006, Jan 12 [Epub ahead of print].

97. Herrmann MG, Durtschi JD, Bromley LK, **Wittwer CT**, Voelkerding KV. Amplicon DNA Melting Analysis for Mutation Scanning and Genotyping: Cross-Platform Comparison of Instruments and Dyes. Clin Chem. 2006 Jan 19; [Epub ahead of print]

98. Willmore-Payne C, Holden JA, Zhou H, Gupta D, Hirschowitz S, **Wittwer CT**, Layfield LJ. Evaluation of HER-2/new gene status in osteosarcoma by fluorescence in situ hybridization and multiplex and monoplex polymerase chain reaction, Arch Path Lab Med, in press, 2006.

BOOK CHAPTERS/REVIEWS:

1. **Wittwer CT**, BW Wyse, RG Hansen. Pantetheine, in: HU Bergmeyer, ed., Methods of Enzymatic Analysis, 3rd Ed., Volume VII, 244-249, Verlag Chemie, Weinheim, 1985.

2. **Wittwer CT**, BW Wyse, RG Hansen. Enzymatic hydrolysis of pantetheine, in: Methods in Enzymology 122:36-43, 1986.

3. **Wittwer, CT**, GB Reed and KM Ririe. Rapid cycle DNA amplification. In K Mullis, F Ferre and R Gibbs (Eds.), The polymerase chain reaction. Springer-Verlag, Deerfield Beach, FL, 174-181, 1994.

4. Brown RA, MJ Lay, and **CT Wittwer**. Rapid cycle amplification for construction of competitive templates, in Genetic Engineering with PCR, (Horton, RM and RC Tait, eds.), Horizon Scientific Press, Norfolk, England, 57-70, 1998.

5. **Wittwer CT**, K Ririe, R. Rasmussen. Fluorescence monitoring of rapid cycle PCR for quantification, in Gene Quantification, Ferre, F., ed., Birkhauser, New York, 129-144, 1998.

6. **Wittwer CT** and MG Herrmman. Rapid thermal cycling and PCR kinetics, in PCR Methods Manual (Innis M, D Gelfand, and J. Sninsky, eds.), Academic Press, San Diego, 211-229, 1999.

7. **Wittwer CT**. Introduction to rapid cycle real-time PCR: methods and applications, in Rapid Cycle Real-Time PCR. S Meuer, **CT Wittwer**, K Nakaguwara, eds., Springer, Berlin, 1-8, 2001.

8. **Wittwer** CT. Introduction to real-time PCR and other homogeneous methods for human DNA analysis, in Molecular Testing in Laboratory Medicine: Selections from Clinical Chemistry, 1998-2001, with Annotations and Updates. DE Bruns, YMD Lo, and CT Wittwer, eds., AACC press, Washington, DC, 3-4, 2002.

9. **Wittwer** CT, PS Bernard, AO Crockett, SD Bohling and KSJ Elenitoba-Johnson. Single nucleotide polymorphism (SNP) genotyping by probe melting curve analysis, in Tumor Markers: Physiology,

Pathobiology, Technology and Clinical Applications. EP Diamandis, HA Fritsche, H Lilja, DW Chan and MK Schwartz, ed., AACC press, Washington, DC, 513-515, 2002.

10. Gundry CN and CT Wittwer. SYBR Green I analysis of the trinucleotide repeat responsible for Huntington's disease. In: Rapid Cycle Real-Time PCR – Methods and Applications: Genetics and Oncology. Dietmaier, **C. Wittwer**, and Sivasubrananian, (eds), Springer, Berlin, 57-63, 2002.

11. Chang CC, S Perkins and **CT Wittwer**. Measurement of MDR1 gene expression by real-time quantitative RT-PCR using the LightCycler instrument. In: Rapid Cycle Real-Time PCR – Methods and Applications: Genetics and Oncology. Dietmaier, **C. Wittwer**, and Sivasubrananian, (eds), Springer, Berlin, 199-205, 2002.

12. **Wittwer CT.** Book review of: RT-PCR Protocols. Methods in Molecular Biology, Vol. 193. Joe O'Connell, ed. Totowa, NJ: Humana Press, 2002, 378 pp. Clin. Chem. 49:345, 2003.

13. **Wittwer CT** and N Kusukawa. Real-Time PCR, in Molecular Microbiology: Diagnostic Principles and Practice. DH Persing, FC Tenover, J Versalovic, YW Tang, ER Unger, DA Relman, TJ White, eds., ASM Press, Washington, DC, 71-84, 2004.

14. **Wittwer** CT, N Kusukawa. Molecular Diagnostics Technology, in Clinical Diagnostic Technology: The Total Testing Process. K Ward-Cook, C Lehmann, L Schoeff, and RH Williams, eds., AACC Press, Washington DC, in press, 2005.

15. **Wittwer** CT, N Kusukawa. Nucleic Acid Techniques, in Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed, Burtis C, Ashwood ER, and Bruns DE, eds, Elsevier Science, Philadelphia, Chapter 37, 1407-1449, 2005.

16. Pryor, R, **CT Wittwer**. Real Time PCR and Melting Curve Analysis. In: Clinical Applications of PCR (Methods in Molecular Biology), Lo YMD, Chiu RWK, and Chan KCA, eds. Humana Press, Totowa NJ, in press, 2005.

17. Gingeras TR, Higuchi R, Kricka, LJ, Lo YMD, **Wittwer CT**. Fifty years of Molecular (DNA/RNA) diagnostics, Clin Chem, 2005;51:661-71.

18. **Wittwer CT.** Book review of: DNA from A to Z, by Daniel H. Farkas. Washington, DC: AACC Press, 2004, 144 pp. Clin Chem 2005 51: 1320-1.

19. Dujols V, Kusukawa N, McKinney JT, Dobrowolsky SF, **Wittwer CT**. High-resolution melting analysis for scanning and genotyping., in Real Time PCR. Tevfik D, ed., Taylor and Francis, Abingdon, in press, 2006.

20. Erali, M, Palais, B, Wittwer, CT. SNP genotyping by unlabeled probe melting analysis. In: Molecular Beacons (Methods in Molecular Biology), Seitz, O and Marx, A, eds., Humana Press, Totwas, New Jersey, 2006.

WEB PRESENTATIONS:

1. Applications of Rapid Cycle Real Time PCR, 2001.
   http://www.aacc.org/education/pcr/default.stm

RECENT ABSTRACTS:

1. Graves CP, **CT Wittwer**, BW Wyse, CT Windham, RG Hansen. Cholesterol-lowering by pantethine is due to cysteamine. Am. J. Clin. Nutr. 43:109A, 1986.

2. Schweitzer CM, **CT Wittwer**, BW Wyse, RG Hansen. Enzymatic liberation of pantothenic acid from whole blood. Am. J. Clin. Nutr. 43:143A, 1986.

3. Graves CP, **CT Wittwer**, MA Peterson, E Jorgenson, CT Windham, BW Wyse, RG Hansen. Pantetheine lipomodulation: mediation by cysteamine in vitro and in vivo. Fed. Proc. 46:1476 (Abstract #6770), 1987.

4. Peterson MA, **CT Wittwer**, E Jorgenson, CT Windham, BW Wyse. The effect of pantothenate status on serum triglycerides and free fatty acids in rats fed a high fat diet. Fed. Proc. 46:1489 (Abstract #6843), 1987.

5. Beck SN, **CT Wittwer**, E Jorgenson, CW Windham, BW Wyse, RG Hansen. Mild pantothenate deprivation raises serum triglycerides, free fatty acids, and beta-hydroxybutyrate in weanling rats. FASEB J. 2:A1204 (Abstract #5280), 1988.

6. Powell RE, WA Knape, CW DeWitt, **CT Wittwer**. Quantitative immune complex assay by flow cytometry using Raji cells. Clinical Applications of Cytometry, Charleston SC, September, 1989.

7. Knape WA, **CT Wittwer**. Quantitative flow cytometric plasma OKT3 assay for monitoring OKT3 therapy in transplant patients. Clinical Applications of Cytometry, Charleston SC, September, 1989.

8. O'Connell JB, KG Renlund, EH Hammond, **CT Wittwer**, RL Yowell, CW DeWitt, KW Jones, WA Gay, RL Menlove, MR Bristow. Sensitization to OKT3: increased frequency with prolonged administration. American College of Cardiology, New Orleans, LA, March, 1990.

9. Chung HT, MJ Youn, SD Hong, WS Ahn, SJ Kim, **CT Wittwer**. Prognostic value of nuclear DNA content in gestational trophoblastic disease by flow cytometry. Cytometry, Supp. 4, 51 (Abstract 333A), 1990.

10. Knowles J, K Rife, **CT Wittwer**. "DNALYSIS" - a new software program for DNA-content cell cycle analysis and report generation. Cytometry, Supp. 4, 88 (Abstract 525B), 1990.

11. **Wittwer CT**, KD Bauer. Use of proteinase K, SDS, and heat to decrease light scatter variation and peak width in DNA-content analysis of formalin-fixed, paraffin-embedded tissue. Cytometry, Supp. 4, 80 (Abstract 487B), 1990.

12. Bagwell B, K Bauer, B Davis, C Hitchcock, T Kute, M Owens, P Rabinovitch, T Shankey, G Stelzer, M Waxdal, **C Wittwer**. Interlaboratory analysis of DNA by flow cytometry. Cytometry, Supp. 4, 82 (Abstract 493B), 1990.

13. Garling DJ, GC Fillmore, **CT Wittwer**. Optimization of time and temperature for denaturation, annealing, and elongation in DNA amplification using hot air cycling. Academy of Clinical Laboratory Physicians and Scientists, San Diego, June, 1990 (**Received Young Investigators Award**).

14. **Wittwer CT**, AD Hernandez, EH Hammond, JM Avent, J Greenwood, AW Middleton, DS Dahl, RG Middleton and JA Smith. Tumor ploidy as a predictive factor in patients with localized prostate cancer. American Urological Association, Toronto, October, 1991.

15. Gumpper KL and **CT Wittwer**. Quantitative dual parameter antiplatelet antibody assay. Clinical Applications of Cytometry, Atlanta, October, 1991.

16. Albro JA and **CT Wittwer**. Proteinase K digestion of formalin-fixed paraffin-embedded liver tissue eliminates false aneuploid peaks and improves histogram quality. Clinical Applications of Cytometry, Atlanta, October, 1991 (**Received Young Investigators Award**).

17. Kershisnik, MM, CCJ Sun, **CT Wittwer**, JM Andrews and AS Knisely. Cytomegalovirus infection, fetal liver disease, and neonatal hemochromatosis. Pediatr. Pathol., 12:243-244, 1992.

18. Hammond, E, **CT Wittwer**, R Yowell, L Hartung, S Olsen, D Taylor, D Renlund. Monitoring of cardiac transplant patients for OKT3 sensitization prevents adverse outcome. International Society for Heart Transplantation. San Diego, April, 1992.

19. Segal, G, **CT Wittwer**, A Fishleder, M Stoler, R Tubbs, C Kjeldsberg. Identification of clonal B cell populations by rapid cycle PCR: a practical screening method for the detection of immunoglobulin gene rearrangements. International Academy of Pathology, Atlanta, March, 1992 (**Received Young Investigators Award**).

20. Segal, G, **CT Wittwer**, A Fishleder, M Stoler, R Tubbs, C Kjeldsberg. Molecular evidence supporting concurrent natural immunoglobulin gene rearrangement and t(14:18) in follicular lymphomas. International Academy of Pathology, Atlanta, March, 1992.

21. Peat, EB, HR Hill, LG Veasy, **CT Wittwer**, CW DeWitt, JB Zabriskie. Flow cytometric analysis of the D8/17 B-lymphocyte marker in rheumatic fever families in Utah. Ped. Research. 31:174A, 1992.

22. Balis, UJ, R Rohr, **CT Wittwer**. High resolution image processing system with standard components. Cytometry, 14:51(424B), 1993.

23. **Wittwer, CT**, MG Herrmann. Alternative reporting of the S-phase fraction in aneuploid breast cancer: recommendations based on 1646 flow cytometry cases. Cytometry, 14:46(380B), 1993.

24. **Wittwer, CT**. Kinetic modeling of DNA amplification. Science Innovations '93, Boston, Mass., platform presentation, Aug. 1993.

25. Campana, CF, **CT Wittwer**. In situ polymerase chain reaction: nonspecific amplification in formalin-fixed paraffin-embedded tissues, Academy of Clinical Laboratory Physicians and Scientists, Los Angeles, June, 1994 (**Received Young Investigators Award**).

26. Lowery, M, CG Sciotto, H Wilson, **CT Wittwer**. Large granular lymphoctyosis with natural killer phenotype: a collection of clinical cases. Clinical Applications of Cytometry, Charleston, SC, Oct., 1994.

27. Swerdlow H, **CT Wittwer**, R Gesteland. Automated high-speed DNA diagnostics in a capillary format. Sixth International Symposium on High Performance Capillary Electrophoresis, San Diego, CA, 1994.

28. Lowery, M, M Issa, L O'Donnell, **CT Wittwer**, A Brothman. Flow cytometric and cytogenetic correlations in patients with minimally differentiated acute non-lymphocytic leukemias (FAB M0). Clinical Applications of Cytometry, Charleston, SC, Oct., 1994.

29. LC Lim and **CT Wittwer**. Mantle cell lymphomas, Academy of Clinical Laboratory Physicians and Scientists, June, 1995 (**Young Investigators Award**).

30. Herrmann M, **C Wittwer**, L Wu. Apolipoprotein E genotyping using rapid two temperature cycle DNA amplification, AACC, 1995.

31. Miya TG, **CT Wittwer**, ER Ashwood. Detection of hepatitis B viral DNA (HBV DNA) with alkaline lysis extraction and one-stage PCR, AACC, 1995.

32. Endo M, PG Beatty, TM Vrede, **CT Wittwer**, S Singh and CJ Parker, Effects of syngeneic bone marrow transplantation without ablative therapy on a patient with paroxysmal nocturnal hemoglobinuria, American Society of Hematology, Seattle, 1995.

33. Hussey CE, M Lay, **CT Wittwer**, and GH Segal. A simple, rapid PCR-based approach for the molecular detection of t(15;17) in acute promyelocytic leukemia (APL). US Academy of Pathology, Augusta, GA, 1996.

34. Brown M and **CT Wittwer**. A systematic study of resonance energy transfer between hybridization probes. Acad. Clin. Lab. Phys. Sci. **Young Investigators Award**, San Diego, June, 1998.

35. J Smith, S Dobrowski, and **CT Wittwer**. Genotyping of medium chain acyl-CoA dehydrogenase deficiency (MCAD) with adjacent fluorescent hybridization probes during PCR. Acad. Clin. Lab. Phys. Sci. **Young Investigators Award**, San Diego, June, 1998.

36. **CT Wittwer**, M Herrmann, D Heap. Temperature Cycling by Adiabatic Compression. Whitaker Biomedical Engineering Foundation, San Diego, Aug. 1998.

37. Chang C, S Perkins and **CT Wittwer**. Measurement of MDR1 gene expression by real time quantitative RT-PCR. US and Canadian Academy of Pathology., San Francisco, March, 1999.

38. Elenitoba-Johnson KSJ, SD Bohling, TC King, and **CT Wittwer**. Multiplex PCR by multicolor fluorimetry and fluorescence melting curve analysis (FMCA) for detection of N-ras mutations. European Association for Haematopathology, London, May, 2000.

39. Greenwood J, M Brown, R Weiss, **C Wittwer** and S Perkins. Flow cytometric detection of biclonal chronic lymphocytic leukemia (CLL). Clinical Cytometry Society, Austin, Sept, 2000.

40. Bernard PS, **CT Wittwer**, L Layfield and J Holden. HER-2/neu and topoisomerase II alpha: linked genes with prognostic and predictive value in invasive ductal cell breast carcinoma. US and Canadian Academy of Pathology, 2001.

41. Bernard PS, **CT Wittwer**, J Holden and L Layfield. HER-2/neu status in breast cancer by real-time quantitative PCR: A comparison of methods. US and Canadian Academy of Pathology, 2001.

42. Bernard PS, H Millward, W Samowitz and **CT Wittwer**. Homogenous amplification and mutation scanning of the P53 gene using fluorescent melting curves. ACLPS, Seattle, 2001.

43. Davidson, RT; Beck, SN; Song, WO; **Wittwer, CT**. Pantothenate-p-nitroanilide as a substrate for continuous spectrophotometric assay of pantetheinase activity. FASEB Journal 15:A964, 2001.

44. Von Ahsen N, **Wittwer CT**, Schutz E. Influence of Mg++, DMSO and dNTPs on melting temperature in real-time PCR based on thermodynamic nearest-neighbor calculations, 33rd Oak Ridge Conference, Seattle, 2001.

45. Garcia-Villalba MP, ND Denkers, **C Wittwer**, RD Nelson, TJ Mauch. Reciprocal changes in AT1 and AT2 angiotensin receptor mRNA expression in the developing rat kidney, Am. Soc. Nephrol.,2002.

46. McKinney J, Dobrowolski SF, and Wittwer CT. Heteroduplex detection with LCGreen and HR-1 high-resolution thermal denaturation instrument, American College of Medical Genetics, San Diego, March 13-16, 2003.

47. Margraf RL, Mao R, Highsmith WE, Holtegaard LM, Wittwer CT. Mutation scanning of the RET proto-oncogene using unlabeled probes and high-resolution melting analysis. Assoc Mol Path, #TT12, Los Angeles, Nov, 2004.

48. Seipp MT, Erali M, Wittwer CT. HLA-B27 typing: evaluation of an allele-specific PCR melting assay and two flow cytometric antigen assays. Assoc Mol Path, #H22, Los Angeles, Nov, 2004.

49. Liew M, Nelson L, Johnson M, Graham R, Meadows C, Erali M, Mao R, Lyon E, Wittwer CT. Fluorescent SNP genotyping by high-resolution melting analysis without probes. Cool Tools and Hot Applications, San Francisco, Nov. 2004.